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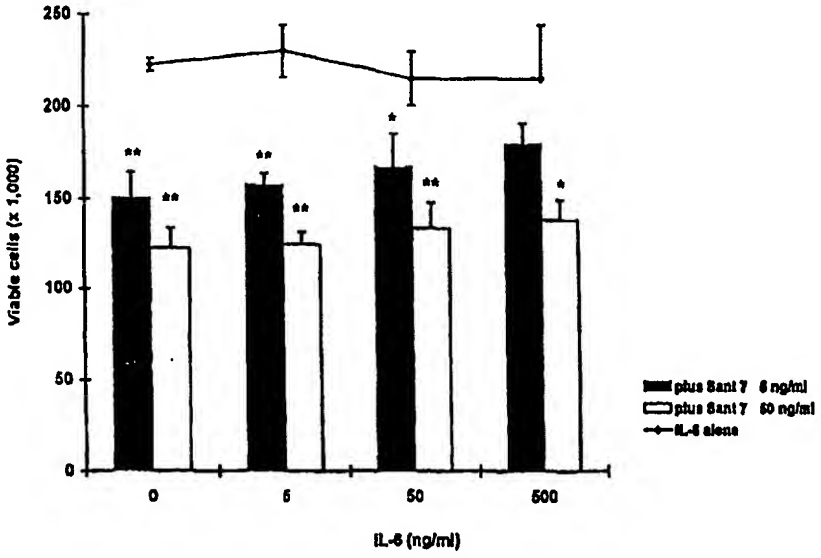

(57) Abstract

The invention refers to compositions containing chemotherapy agents frequently used in cancer therapy, such as etoposide and cisplatin, in combination with an inhibitor of the interleukin 6 receptor complex signalling by gp130. Such compositions decrease the drug resistance of a tumour cell line. A specific embodiment of the invention uses a superantagonist of interleukin 6, the mutein of IL-6, Sant 7 and reported in the sequence listing as SEQ ID NO:1, which is capable of binding the sub-unit IL6-Rα of the IL-6 receptor. Sant 7 indicated inhibits binding, by steric blocking, with the sub-unit gp130 of the IL-6 receptor complex. Transduction of the signal from this cytokine is in fact involved in the multiple drug resistance mechanisms that are typically developed by tumour cells following chemotherapy.

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ANTI-TUMOUR PHARMACEUTICAL COMPOSITIONS CAPABLE OF REDUCING DRUG RESISTANCE IN TUMOUR CELLS

DESCRIPTION

The present invention relates to pharmaceutical compositions with anti-tumour activity that are also capable of decreasing tumour drug resistance. More specifically the invention relates to the use of mutant molecules of human interleukin 6 for preparation of chemotherapy mixes designed to treat human prostate carcinoma.

Cancer treatment currently in use includes at least two strategies: surgery and/or chemotherapy. Which of these strategies is used generally depends on the tumour being treated. In some cases, other treatments such as X-ray therapy and cobalt therapy are associated with the treatment indicated above. Generally speaking, all patients undergo chemotherapy associated with various strategic treatments.

Chemotherapy usually involves the use of compositions containing a number of molecules with cytotoxic activity. This results in various problems connected to the intrinsic toxicity of the drugs, to the lack of specificity in selecting a tumour cell rather than a "normal" one and furthermore to the response of the tumour cell. A tumour cell has undergone a series of structural and genetic modifications that make it different from a normal cell. A cancer cell, for example, has altered its proliferation system, so that it is capable of invading and attacking tissues and organs, and is also capable of "avoiding" the control of the immune system. A tumour mass responds to drug treatment by continuing to select drug resistant cells. For this reason, the ability to kill a tumour cell is connected with the survival systems that are typical of each primary tumour cell and of the cells that are subsequently selected. This is the reason why current cancer treatment provides for treatment using a mixture

of chemotherapy drugs, which can result in any case ineffective in the treatment of certain types of tumour form.

Drug resistance is in fact almost always the cause and the effect of progression in a tumour that does not respond to treatment. The phenomenon of multiple drug resistance (MDR) is in fact among the basic problems in cancer treatment, which has been dealt with in two ways: on the one hand by studying the resistance mechanisms, on the other hand by using drug preparations consisting of a number of molecules capable of preventing the tumour cell from establishing resistance to a specific drug.

Although some of the proteins responsible for this resistance have been identified, the phenomenon is still very poorly understood. However, a series of experiments have proven that the molecular mechanisms of MDR form part of a cell response similar to the cell response to hormones, growth factors or cytokines, which can generally speaking be indicated as signals external to the cell.

In particular it has been observed that certain cytokines and growth factors, as well as their receptors, are effectively involved in some of the mechanisms of drug resistance of certain tumour forms. Some (for example G-CSF or GM-CSF) have the effect of increasing the death of tumour cells when adiminstered in association with drugs, some others (for example interleukin 6) have instead the effects of increasing the survival of the tumour cells in many types of cancer forms, one of the most important of which is prostate cancer.

Prostate carcinoma is in fact the most common form of tumour diagnosed in males, which in 1995 was the tumoural form that with the exception of the lung cancer, has reached the highest mortality rate in the United States. Although radical prostatectomy can be used to treat patients with localised tumours, the majority of

patients show an advanced stage of the disease at the time of diagnosis. In any case it has been observed that although systematic androgen ablation treatment may be of use when treating patients suffering from prostate cancer, 12-18 months from the start of treatment tumour cells that are independent of the hormones develop. This specific type of tumour form is usually particularly resistant to chemotherapy.

In effect, it has been demonstrated that prostate cancer cell lines, whether or not they are hormone-dependent, have the ability to secrete interleukin 6 (IL-6) whose involvment in the progression of various tumour forms has been observed.

IL-6 is in fact a multifunctional molecule with a wide range of action in the stimulation or inhibition of cell growth (depending on the type of target cell). It is also involved in certain immune mechanisms, and can also have an autocrine or paracrine effect as a growth factor in certain tumours such as multiple myeloma. IL-6 is a factor protecting against cell death in multiple myeloma cells, and inhibits desamethasone-induced apoptosis. It has been observed that IL-6 blocks the cytotoxicity of some anti-cancer agents and biological factors in myeloid leukemic cells.

With regard to human prostate cancer cell lines it has recently been observed that both in the cell line PC-3 and DUI145 the secretion of IL-6 is autocrine and involves super-induction of cell growth. In these cells therefore IL-6 represents a resistance factor for cytotoxicity induced by drugs such as etoposide and cisplatinum (hereinafter referred also as VP-16 and CDDP respectively). Furthermore, the production of IL-6, apart from its role in the tumour's growth, can have an undesired effect on the bone metastases, inducing osteoclast activity and cachexia in the host.

Furthermore, in all the cases where the androgens suppress the production of IL-6 at a transcriptional

level, in cells that are not responsive to androgens, the production of IL-6 is up-regulated.

The IL-6 receptor is a heterodimeric glycoprotein that has been fully identified at molecular level. It is made up of a receptor for the ligand IL6-Rα and a transducer for the signal gp130. IL-6 promotes sequential formation of a complex between the two molecules: IL6-Rα binds IL-6 with high affinity and then forms a complex with gp130 that is crucial for signal transduction. This proceeds in cascade to activate tyrosine kinases of the Jak family.

It has also been observed that the receptor complex induced by IL-6 is related to those of other cytokines, such as the leukemia inhibitory factor (LIF), the ciliary neurotrophic factor (CNTF), interleukin-11 (IL-11), cardiotrophin 1 (CT-1) and oncostatin M (OM), which all require gp130 as signal transducing subunit.

Oncostatin M is a 28 kD glycoprotein secreted by activated macrophages and T lymphocytes, originally described for its ability to inhibit the growth of human melanoma A375 cells and also active on other tumour cell lines. However, this factor can also stimulate cell growth, by an autocrine mechanism, in AIDS related Kaposi sarcoma cells and is capable of eliciting a multitude of other biological effects. As reported above, the signal is transduced by the protein gp130.

As the existence of specific receptors to oncostatin M in PC-3 cells has been reported, the inventors studied the behaviour of the PC-3 cell line, derived from a prostate carcinoma, with respect to IL-6 and OM activity. More specifically, the following was investigated:

1) whether PC-3 synthesise and secrete OM;

2) whether OM, like IL-6, exerts an effect on PC-3 tumour cell growth;

3) whether OM, like IL-6, regulates tumour cell resistance to cytotoxic drugs like VP-16 and CDDP;

4) whether IL-6 antagonists or inhibitors of gp130-mediated signalling affect cell survival and drug resistance.

Unexpectedly, the results of the above investigations showed that the combination of etoposide and/or cisplatin with a protein molecule capable of blocking the formation of the interleukin 6 receptor complex and/or the OM receptor complex, increases the drug sensitivity of the cell line, probably by the decrease of cell drug-resistance.

More specifically, the present invention is based on experimental observation of the PC-3 cells, in which oncostatin M, like IL-6, was shown to play an important part in regulation of the cell proliferation and in drug resistance. The inventors have in particular demonstrated that using the gp130 as a transducer, OM can regulate survival and resistance of prostate cancer cells, and can act as a possible paracrine factor, mimicking and amplifying the effects of IL-6, which is likewise produced in the cell-mediated response of the immune system.

With regard to IL-6, it was previously demonstrated that IL-6 increases drug resistance in a human prostate carcinoma cell line (Borsellino N. et al. 1995).

The observations made on IL-6 and OM do not exclude the possibility that these factors may have a positive effect also on survival of the tumour cells, preventing necrosis or drug-induced apoptosis. It is also not excluded that IL-6 or OM can have a specific influence on a biochemical drug adaptation mechanism, as was ascertained in kidney tumour cells, that glutathione S-transferase (one of those enzymes that intervene in drug resistance) is over-regulated by IL-6.

However, independently of the mechanism by which IL-6 and OM induce drug resistance, which in any case is not known, the inventors have tested the effects of their inhibition on cell proliferation. Therefore the inventors

have first tested the capability of inhibitors of both molecules, of blocking cell growth. In particular the inhibitors tested were the IL-6 mutein Tyr31Asp, Gly35Phe, Leu57Asp, Glu59Phe, Asn60Trp, Gln75Tyr, Ser76Lys, Ser118Arg, Val121Asp, Gln175Ile, Ser176Arg, Gln183Ala, hereinafter referred to as Sant 7 (disclosed in the patent application WO 96/34104 and represented in the sequence listing below as SEQ ID NO:1) and antibodies anti-IL-6 as inhibitors of IL-6 mediated effects, and an antisense oligonucleotides in the sequence coding for gp130 represented as SEQ ID NO:2 and SEQ ID NO:3 in the sequence listing below, as inhibitor of both IL-6 and OM, through the blockage of gp130.

Anti-IL-6 antibodies and the IL-6 superantagonist Sant 7, results in fact in inhibition of cell growth and in increasing the sensitivity of PC-3 to the chemotherapy drugs VP-16 and CDDP by the neutralisation of IL-6 activity. The antisense oligonucleotide was demonstrated to inhibit the effects of IL-6 and of OM on the tumour cells, probably by blocking the signal transduction process.

This last result is particularly significant, as the expression of gp130 in prostate cancer cells provides a mechanism by which the tumour cells can respond to a whole family of cytokines binding gp130. As a consequence of this fact, and as a consequence of the fact that the experiments carried out in connection with this invention show that IL-6 and OM have a similar effect on the tumour cells, it can be assumed that other members of the same family may have a similar effect. Thus in reality as prostate cancer is under the control of a number of cytokines that all have in common gp130, the capability of blocking it could be particularly relevant. In any case in effect, if expression of gp130 or its transducer activity is blocked in any way, the effect of IL-6 or of OM on the cells is also blocked.

The present invention consisting in pharmaceutical compositions containing at least one of the above mentioned inhibitors in combination with at least one chemotherapic drug, provides a new approach in cancer treatment based on the capability of counteracting the development of refractiveness to the chemotherapy drugs.

The use of preparations involving anti-IL-6 antibodies can be associated with severe side effects, including stabilisation of the IL-6 in circulation, with a consequent increase in the average life of the cytokine. In this direction IL-6 antagonists such as Sant 7 can be of great therapeutic use. It is also true that the effects of IL-6 and OM might be prevented by the use of anti-gp130 antisense oligonucleotides, or of inhibitors of the tyrosine kinases involved in the biochemical route for signal transduction by gp130.

Subject of the present invention is a pharmaceutical composition capable of reducing the cell drug resistance to anti-tumour drugs, comprising at least one chemotherapeutic drug and at least one agent capable of inhibiting the transduction of the signal sent by a cytokine having the protein gp130 as transducer and a pharmaceutically acceptable carrier.

In particular subject of the present invention is a pharmaceutical composition in which the chemotherapeutic drugs are etoposide, or analogs thereof, and cisplatin, or analogs thereof. In the case in which the drugs are etoposide and cisplatin, particular importance is taken on by the composition in which the agent capable of inhibiting the transduction of the signal sent by a cytokine having the protein gp130 as transducer is the IL-6 mutein represented by SEQ ID NO:1, or antisense oligonucleotides, fragments or derivatives thereof, in the sequence coding for gp130.

Further subject of the present invention, is the use of the pharmaceutical compositions above for the preparation of anti-tumour drugs, and for the preparation

of drugs having therapeutic effects on human prostate carcinoma.

Another subject of the present invention is a composition of matter, characterised by the fact of comprising etoposide, cisplatin and the IL-6 mutein represented by SEQ ID NO:1.

The invention will be described in greater detail with reference to the enclosed figures.

Description of the figures

Figure 1 shows the effects of stimulation of cell proliferation by exogenous oncostatin M on the PC-3 cell line after 24 hour incubation, as measured by trypan blue dye exclusion test. The results represent the mean ± SD of three different experiments performed in duplicate. A single asterisk indicates $P < 0.05$ with respect to the control; a double asterisk indicates $P < 0.01$ with respect to the control.

Figure 2A shows the effects of IL-6 in combination with monoclonal neutralising antibody anti-human IL-6 on growth of PC-3 cell line after 24 hour incubation, as measured by trypan blue dye exclusion test. The results represent the mean ± SD of three different experiments performed in duplicate. A single asterisk indicates $P < 0.05$ with respect to administration of IL-6 alone, the double asterisk indicates $P < 0.01$ with respect to administration of IL-6 alone.

Figure 2B shows the effects of IL-6 in combination with the IL-6 mutein Sant 7, on growth of PC-3 cell line after 24 hour incubation, as measured by trypan blue dye exclusion test. The results represent the mean ± SD of three different experiments performed in duplicate. A single asterisk indicates $P < 0.05$ with respect to administration of IL-6 alone, the double asterisk indicates $P < 0.01$ with respect to administration of IL-6 alone.

Figure 3 shows the effects of administration of Sant 7 alone, or in combination with oncostatin M on the PC-3

cell line after 24 hours incubation, as measured by trypan blue dye exclusion test. The results represent the mean ± SD of three different experiments performed in duplicate.

Figure 4 shows the effects of administration of antisense oligonucleotides against gp130 (added at a concentration of 10 μM at 0 h and of 5 μM at 24 and 48 h) and of its mismatched mutant in combination with oncostatin M (19 ng/ml) on PC-3 cell line after 72 hours incubation, as measured by trypan blue dye exclusion test. The results represent the outcome ± SD of an experiment performed in triplicate. A repeat experiment gave very similar results. The abbreviation ASO indicates antisense oligodeoxynucleotide against gp130; the abbreviation MUT indicates mismatched mutant of the antisense oligodeoxynucleotide.

PC-3 cells

The human hormone-independent prostate carcinoma PC-3 cell line was developed by Dr. Arie Belldegrun of the University of California at Los Angeles, Los Angeles, CA. PC-3 cells are available from the American Type Culture Collection, with the entry reference number ATCC CRL 1435. The cells were maintained in complete RPMI 1640 medium (HiClone Laboratories, Logan, UT, USA) supplemented with 10% heat-inactivated fetal calf serum (HyClone), 1% L-glutamine (HyClone), 1% pyrovate (HyClone), and 1% penicillin/streptomycin solution containing 10^4 u/ml penicillin G and 10 mg/ml streptomycin sulphate. The cells were grown as adherent cells in a humidified atmosphere at 37°C in 5% carbon dioxide.

When the tumour cells were used experimentally, they were first treated with trypsin-EDTA, washed and resuspended in complete medium.

OM ELISA test

The presence of intracellular and extracellular OM protein was determined by ELISA. Briefly, 5 x 10^5 cells

were incubated in complete medium; after 48 hours the cells were treated with trypsin-EDTA and harvested, and the supernatant was separated from the cells by centrifugation. Cells and supernatant were collected to measure the OM content. The cells were resuspended in 1 ml complete medium and the viable cells were counted by the trypan blue dye exclusion test and sonicated. After centrifugation the medium was collected to measure the intracellular content of OM. The determination of both extracellular and intracellular levels of OM was performed by an OM ELISA kit supplied by Amersham (Little Chalfont, England).

Cytotoxicity assay

The effects of OM, the anti-human IL-6 antibody, Sant 7, genistein (GNS), the antisense oligonucleotide against gp130 on cytotoxicity, and the effects of the drugs alone or in combination were assessed by the trypan blue dye exclusion test.

The PC-3 cell line was resuspended in complete medium at a concentration of 1 x 10^5 cells/ml after verifying their viability. Amounts of 1 ml of cell suspension were distributed into each well of a 24-well culture plate (Nunc, Roskilde, Denmark). After one night the cells were in a state of adhesion. At time 0 h, the culture medium was replaced with 1 ml of fresh complete medium and reagents were added at appropriate concentrations.

In some experiments testing the growth effect of OM alone, the culture medium was not replaced after overnight incubation and the drugs were added directly to the old medium.

In the experiments testing the association of the oligonucleotides with OM and VP16 the latter were added after 6 h. After 24 hours (and after 72 hours for experiments in combination with other drugs) the cells were harvested by treatment with trypsin. Cell viability was determined by the ability of cells to exclude trypan

blue dye. The cells were assessed visually by microscopy, and the number of viable cells was calculated by subtracting the number of cells that stained positive from the total number of cells. The surviving fraction (s.f.) is obtained from the ratio of the number of experimental viable cells compared to the number of control cells. The percent control cells is calculated by multiplying (s.f.) x 100.

Materials used in the cytotoxicity assay

The drugs used, VP16 and CDDP, were purchased from Bristol-Myers Squibb and stored at 4°C as 0.5 mg/ml and 20 mg/ml solutions. The drugs, which were used at different concentrations, were added at various times to the cell cultures alone, or in combination with the other molecules used in the present invention.

OM (recombinant E. coli, with s.a. 5 x 10^5 units/mg) was purchased from Genzyme (Cambridge, MA, USA). After reconstitution in sterile distilled water to a 1 µg/ml solution, the cytokine was stored at -40°C. OM was added to the cell cultures alone at a concentration of 0.5 ng/ml and 10 ng/ml, or in combination with VP-16 (at concentrations of 0.1 mg/ml, 1 mg/ml, and 2 mg/ml), or with CDDP (at the same concentrations as VP-16), or with Sant 7 (at concentrations of 5 and 50 ng/ml), or with the antisense oligonucleotide against the sequence coding for gp130.

The oligonucleotides SEQ ID NO:2 and SEQ ID NO:3 were designed according to the sequence of cDNA coding for human gp130 as reported by Hibi et al. The oligonucleotides were synthesized and purified by gel filtration by Cruachem Ltd. (Glasgow, UK). After reconstitution in RPMI 1640 at a concentration of 1 mM, they were stored at 4°C. The oligonucleotides were added directly to the culture medium at 0 h (10 mM), at 24 hours and 48 hours (5 mM) alone or after 6 hours in combination with OM (100 ng/ml) or VP16 (0.1 mg/ml and 0.5 mg/ml).

Sant 7 instead, was produced as previously reported (WO 96/34104). After reconstitution in PBS, it was stored at 4°C at a concentration of 6.4 mg/ml. Sant 7 was added to the cell cultures at time 0 alone or in combination with IL-6 (5 ng/ml, 0.5 ng/ml and 1 ng/ml), or VP16 (0.1 mg/ml and 1 mg/ml).

Mouse monoclonal neutralising antibody anti-human IL-6 (clone: B-E8) was purchased from Biosource International (Camarillo, CA, USA) as a PBS solution. Aliquots (10mg/ml) were prepared and stored at -40°C, and added to the cell cultures alone or in combination with IL-6 (5 ng/ml, 50 ng/ml and 500 ng/ml) after dilution in complete medium up to the concentration of 1 mg/ml and 0.1 mg/ml.

Genistein (GNS, purchased from Sigma Chemical Co. - St. Louis, MO, USA) was reconstituted in a DMSO solution to a concentration of 5 mM and frozen at -20°C. GNS was also added alone at time 0 h or in combination with VP16 (0.1 mg/ml and 1 mg/ml).

Evaluation of effectiveness of combined treatments

According to Aapro (ref.), the extent of the combined effect of a number of drugs was evaluated for additivity with respect to the expected result, expressed as the product of the surviving cell fractions, observed with the agents alone, multiplied by 100. In this way, the effect of the combination of a number of drugs can be considered synergistic or antagonistic when the result observed is greater than expected or lower than expected, respectively.

The present invention has been described in general terms up to this point. With the aid of the following examples, a more detailed description of specific embodiments thereof will now be given, in order to give a clearer understanding of the aims, characteristics, advantages and operating methods of the same.

EXAMPLE 1

Effect of OM on PC-3 cells

OM marked with the isotope ^{123}I, made it possible to ascertain that cultivated PC-3 cells bind OM with a binding specificity equivalent to 7.7 fmoles/10^6 cells. The inventors observed that there was no secretion of OM by the PC-3 cells, as assessed by performing an ELISA test in the supernatant of cultivated cells, and the PC-3 cell lysates also did not appear to contain OM. The effect of OM on PC-3 cell growth was then examined. A dose dependent stimulation of cell growth was observed, and a plateau was obtained at a concentration of around 1 ng/ml. As this effect was demonstrated even when the culture medium was replaced, it can be assumed that the OM receptors present on the PC-3 cells are functional and that OM potentiates cell growth (fig. 1). The effect of OM on the sensitivity of PC-3 to drugs of known cytotoxicity such as VP-16 and CDDP was then examined. It was observed that the addition of OM significantly potentiated resistance of PC-3 to both VP-16 and CDDP (tables 1 and 2). Furthermore the extent of resistance was dependent on the concentrations of both OM and the drugs. In connection with the present invention it has been seen that OM amplifies the autocrine effect of IL-6 on cell growth and on drug resistance of PC-3, which suggests that the signals from the two cytokines activate a common transduction mechanism.

Table 1 Effects of combination of OM and CDDP on PC-3 cell line.

CDDP (μg/ml)	% of control growth obtained with		
	CDDP	Plus OM 0.5 ng/ml	Plus OM 10 ng/ml
0		120.3 ± 2.0	131.9 ± 9.2
0.1	99.4 ± 7.1	116.8 ± 9.9 [119.6]	125.5 ± 0.5 [131.1]
1	71.0 ± 7.5	105.8 ± 5.6 [85.4]	100.0 ± 2.0 [93.6]
2	55.1 ± 6.1	88.4 ± 2.0 [66.3]	97.1 ± 8.5 [72.7]

Data are the mean ± SD from three different experiments performed in duplicate. The numbers in parenthesis show the extent of cell survival expected from the effects of OM and CDDP used alone.

Table 2. Effect of combination of OM and VP-16 on growth of PC-3 tumor cells.

VP-16 (μg/ml)	% of control growth obtained with		
	VP-16	VP-16 + OM 0.5 ng/ml	VP-16 + OM 10 ng/ml
0		114.9 ± 2.4	126.3 ± 4.4
1	51.0 ± 6.6	70.2 ± 2.2 [58.7 ± 8.7]	89.3 ± 2.3 [64.6 ± 9.5]*
2	25.8 ± 0.8	51.7 ± 3.3 [29.8 ± 0.9]**	78.0 ± 6.6 [32.7 ± 1.6]**

The cells were seeded at a concentration of 1×10^5 cells/ ml/ well in 24-well culture plates. The cells were allowed to adhere overnight. At time 0, the reagents were added at appropriate concentrations. After 24-h incubation, the cells were harvested by treatment with trypsin-EDTA. Cell viability was determined by the ability of cells to exlude trypan blue dye. Data are the mean ± SD from three different experiments performed in duplicate. The numbers in parentheses show the extent of cell survival expected from the effects of OM and VP-16 used alone. For more details, see Materials and Methods.

* $P < 0.05$ observed results versus expected results.

** $P < 0.01$ observed results versus expected results.

Example 2

Blocking of OM and IL-6 mediated signalling in PC-3 cells with gp130 antisense oligonucleotide

Since both OM and IL-6 share the signal transducing gp130 receptor chain, the activity of these two cytokines should be bloked in absence of gp130. PC-3 cells were trated with gp130 antisense and tested for cell viability. The addition of antisense inhibited cell growth and cell viability by about 20% confirming the positive role of endogenous self secreted IL-6 in cell survival. The co-addition of antisense and OM also inhibited OM-mediated stimulation of PC-3 cell growth (see figure 4). Since IL-6 is a resistance factor in PC-3 cells, blocking of IL-6 signalling by antisense should augment tumour cell sensitivity to cytotoxic drugs. Indeed, as shown in Table 3, treatment of PC-3 with gp130antisense increased the sensitivity of the tumour cells to cytotoxicity by VP-16.

Table 3. Combination cytotoxicity of antisense oligodeoxynucleotide against gp130 and VP-16 in PC-3 cell line.

VP-16 (µg/ml)	% of control growth obtained with:	
	VP-16	Plus antisense oligodeoxynucleotide
0		86.4 ± 4.5
0.1	77.7 ± 0.5	62.1 ± 2.7 [67.3]
0.5	65.0 ± 4.1	43.7 ± 6.9 [56.1]

Data are the mean ± SD of three different experiments performed in duplicate. The numbers in parenthesis show the extent of cell survival expected from the effects of AS and VP-16 used alone.

EXAMPLE 3

Effect of IL-6 and Sant 7 on PC-3 cells

To ascertain whether or not the transduction mechanism is the same, the inventors examined the effect of an IL-6 superantagonist, Sant 7 on the IL-6 and OM-mediated effect. Sant7 is a genetically modified IL-6 mutant, which is characterised by a 70 fold increase in binding affinity to IL6-Rα, but which is not capable of binding gp130. The failure to form the IL6-Rα and gp130 complex blocks signal transduction, so that antibodies directed against IL6, but also the superantagonist Sant7, can be expected to block the IL-6-mediated effects but not to prevent OM from binding its own receptor complex and therefore cannot be expected to prevent transduction of the OM-induced stimulus. The concentration of IL-6 secreted by PC-3 is approximately 0.15 ng/ml in 24 hour cultures.

Using anti-IL-6 antibodies at a concentration of 0.5 nM or 5 nM the growth of PC-3 is inhibited (Fig. 2A and 2B). In particular, the anti-IL-6 antibodies at a concentration of 0.5 nM are not sufficient to completely antagonise the IL-6 present at a concentration of 0.2 nM (Fig. 2A). Similarly, the antagonist activity of monoclonal anti-IL-6 antibodies at a concentration of 5 nM is substantially reduced at IL-6 2 nM. These results demonstrate that a molar ratio of anti-IL-6 antibodies to IL-6 greater than 2.5 is required for full antibody antagonist activity. On the contrary the superantagonist Sant 7 at a concentration of 0.2 nM is effective with respect to 2 nM of IL-6, and a concentration of 2 nM is effective with respect to 20 nM of IL-6. In view of these experiments the effective ratio of Sant7:IL-6 is 1:10 (Fig. 2B).

In a similar manner to that used with anti-IL-6 antibodies to show that IL-6 is a resistance factor in PC-3, an experiment was carried out using Sant7, confirming the experimental data obtained with the

neutralising antibodies.

Sant 7 significantly potentiates the sensitivity of PC-3 to VP16 cytotoxicity in a concentration dependent manner (table 4).

Table 4. Effects of combination of SAnt 7 and VP-16 on PC-3 cell line.

VP-16 (μg/ml)	% of control growth obtained with:		
	VP-16	Plus SAnt 7 1 ng/ml	Plus SAnt 7 5 ng/ml
0		80.0 ± 3.3	72.5 ± 4.5
0.1	90.5 ± 0.7	55.5 ± 2.5 [72.4]	45.5 ± 5.3 [65.3]
1	62.5 ± 4.3	25.2 ± 7.7 [50.0]	25.0 ± 3.2 [45.3]

Data are the mean ± SD from three different experiments performed in duplicate. The numbers in parenthesis show the extent of cell survival expected from the effects of Sant 7 and VP-16 used alone.

EXAMPLE 4

Formulation of pharmaceutical compositions with anti-cancer activity and capable of reducing drug resistance

By extrapolation of the experimental results obtained according to the present invention, a typical formulation to be used to prepare a pharmaceutical composition containing VP16 and CDDP, or more powerful derivatives of said molecules, should be as follows:

Ingredients	Amounts
VP 16	1÷2 mg/ml
CDDP	same amounts as VP 16
Sant 7	1÷5 ng/ml

As well as not excluding substances similar to VP16 and CDDP, the preparation should not necessarily be administered according to the protocols devised for VP16 and CDDP, since the use of Sant 7 decreases drug-resistance. Furthermore the amount of Sant 7 used in example 3 of the present invention should be adjusted in relation to the body weight by comparison with the data for use of VP16 and CDDP, as amounts expressed in grams per kilogram body weight.

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SEQUENCE LISTING

GENERAL INFORMATION

(i) APPLICANT:

ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P.ANGELETTI S.p.A.

(ii) TITLE OF INVENTION: "ANTI-TUMOUR PHARMACEUTICAL COMPOSITIONS CAPABLE OF REDUCING DRUG RESISTANCE IN TUMOUR CELLS"

(iii) NUMBER OF SEQUENCES: 3

(iv) MAILING ADDRESS:

(A) ADDRESSEE: Societa' Italiana Brevetti

(B) STREET: Piazza di Pietra, 39

(C) CITY: Rome

(D) COUNTRY: Italy

(E) POST CODE: I-00186

(v) COMPUTER-READABLE FORM:

(A) TYPE OF SUPPORT: Floppy disk 3.5'' 1.44 MBYTES

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS Rev. 5.0

(D) SOFTWARE: Microsoft Word 6.0

(viii) AGENT INFORMATION

(A) NAME: DI CERBO Mario (Dr.)

(B) REFERENCE: RM/X88878/PC-DC

(ix) TELECOMMUNICATIONS INFORMATION

(A) TELEPHONE: 06/6785941

(B) TELEFAX: 06/6794692

(C) TELEX: 612287 ROPAT

(1) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 184 aminocid

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FURTHER CHARACTERISTICS

(A) NAME: Sant7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Pro Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg
1               5                  10                  15

Gln Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Asp Ile
            20                  25                  30

Leu Asp Phe Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn
        35                  40                  45

Met Cys Glu Ser Ser Lys Glu Ala Asp Ala Phe Trp Asn Leu Asn Leu
    50                  55                  60

Pro Lys Met Ala Glu Lys Asp Gly Cys Phe Tyr Lys Gly Phe Asn Glu
65                  70                  75                  80

Glu Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val
                85                  90                  95

Tyr Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala
            100                 105                 110

Arg Ala Val Gln Met Arg Thr Lys Asp Leu Ile Gln Phe Leu Gln Lys
        115                 120                 125

Lys Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn
    130                 135                 140

Ala Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp
145                 150                 155                 160

Met Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Ile Arg
                165                 170                 175

Ser Leu Arg Ala Leu Arg Ala Met
            180
```

(2) INFORMATION ON SEQUENCE SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH:18 bp

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(iv) ANTI-SENSE: yes

(ix) FEATURE:

(D) OTHER INFORMATION: sequence from cDNA coding for human gp130

(xi) SEQUENCE DESCRIPTION SEQ ID NO: 2:

CCA AGT CTG CAA CGT CAA

1 10

(3) INFORMATION ON SEQUENCE SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 18 bp

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(iv) ANTI-SENSE: yes

(ix) FEATURE:

(D) OTHER INFORMATION: sequence from cDNA coding for human gp130

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTA ACT CTG CAG CGG CAA

1 10

CLAIMS

1. A pharmaceutical composition capable of reducing drug resistance to anti-tumour drugs, comprising at least one chemotherapeutic drug and at least one agent capable of inhibiting the transduction of the signal sent by a cytokine having the protein gp130 as transducer and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to claim 1, in which the chemotherapeutic drugs are etoposide, or analogs thereof, and/or cisplatin, or analogs thereof.

3. The pharmaceutical composition according to claim 2, in which the drugs are etoposide and/or cisplatin and the agent capable of inhibiting the transduction of the signal sent by a cytokine having the protein gp130 as transducer is the IL-6 mutein represented by SEQ ID NO:1.

4. The pharmaceutical composition of claim 1, in which the agents capable of inhibiting the transduction of the signal sent by a cytokine having the protein gp130 as transducer are antisense oligonucleotides, fragments or derivatives thereof, in the sequence coding for gp130.

5. Use of pharmaceutical compositions according to anyone of the preceding claims for the preparation of anti-tumour drugs.

6. Use of the pharmaceutical composition according to claim 3 for the preparation of drugs having therapeutic effects on human prostate carcinoma.

7. A composition of matter, characterised by the fact of comprising etoposide, cisplatin and the IL-6 mutein represented by SEQ ID NO:1.

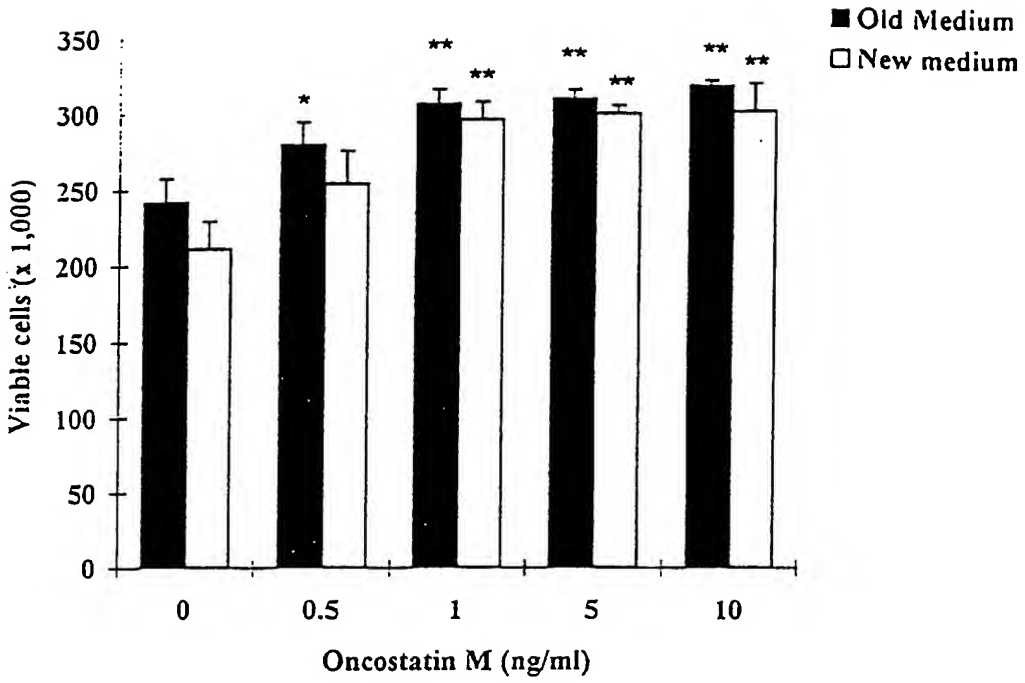
Old Medium
New medium
Vinble cells (x 1,000)
350
300
250
200
150
100
50
0
0
0.5
1
5
10
Oncostatin M (ng/ml)
*
**

FIG. 1

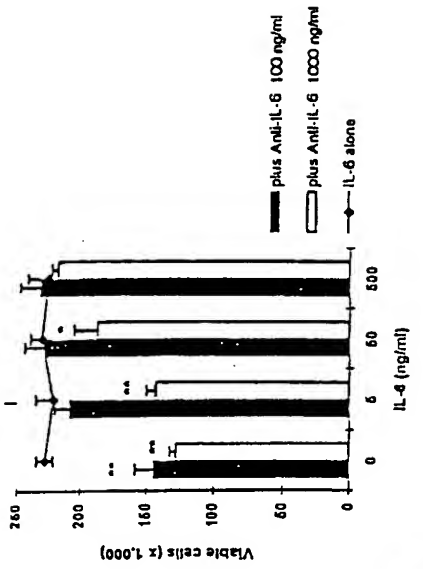
plus Anti-IL-6 100 ng/ml
plus Anti-IL-6 1000 ng/ml
IL-6 alone
Viable cells (x 1,000)
IL-4 (ng/ml)

FIG. 2A

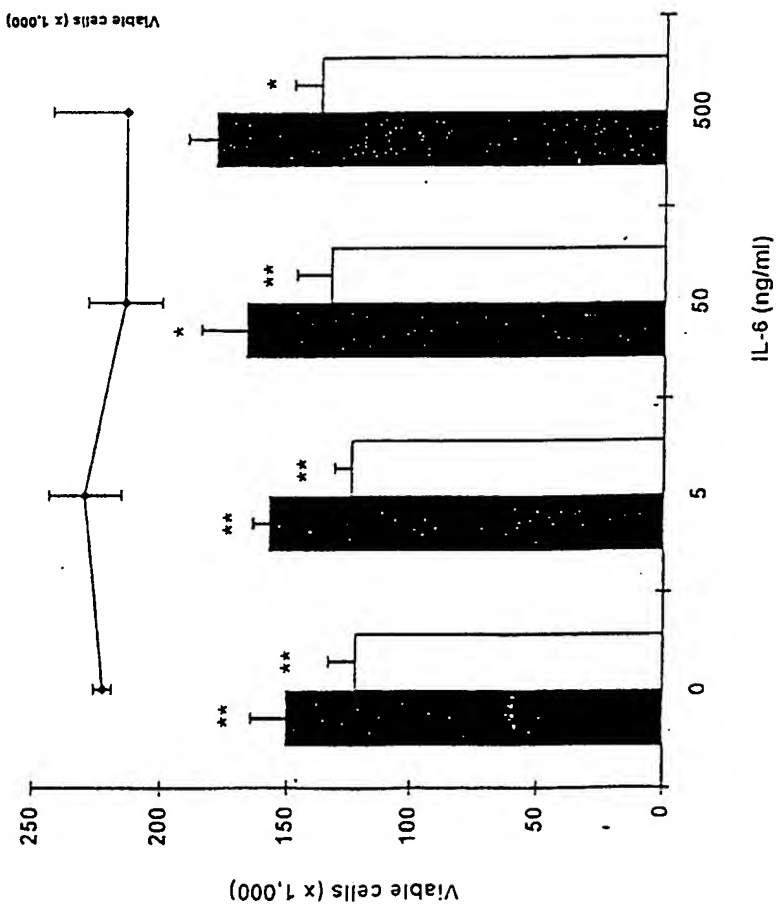
plus Sant 7 5 ng/ml
plus Sant 7 50 ng/ml
IL-6 alone
Viable cells (x 1,000)
IL-6 (ng/ml)

FIG. 2B

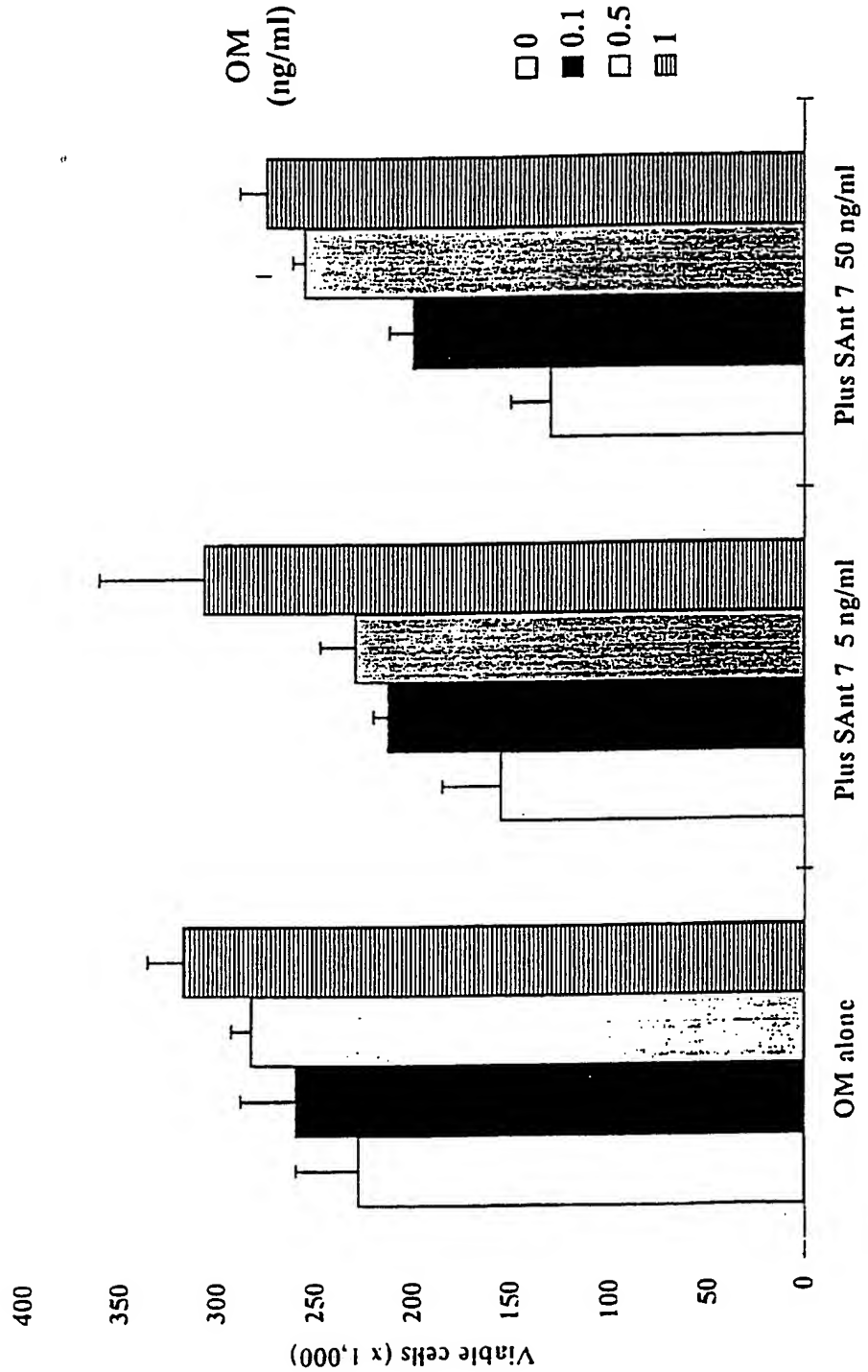
OM (ng/ml)
0
0.1
0.5
1
Viable cells (x 1,000)
400
350
300
250
200
150
100
50
0
OM alone
Plus SAnt 7 5 ng/ml
Plus SAnt 7 50 ng/ml

FIG. 3

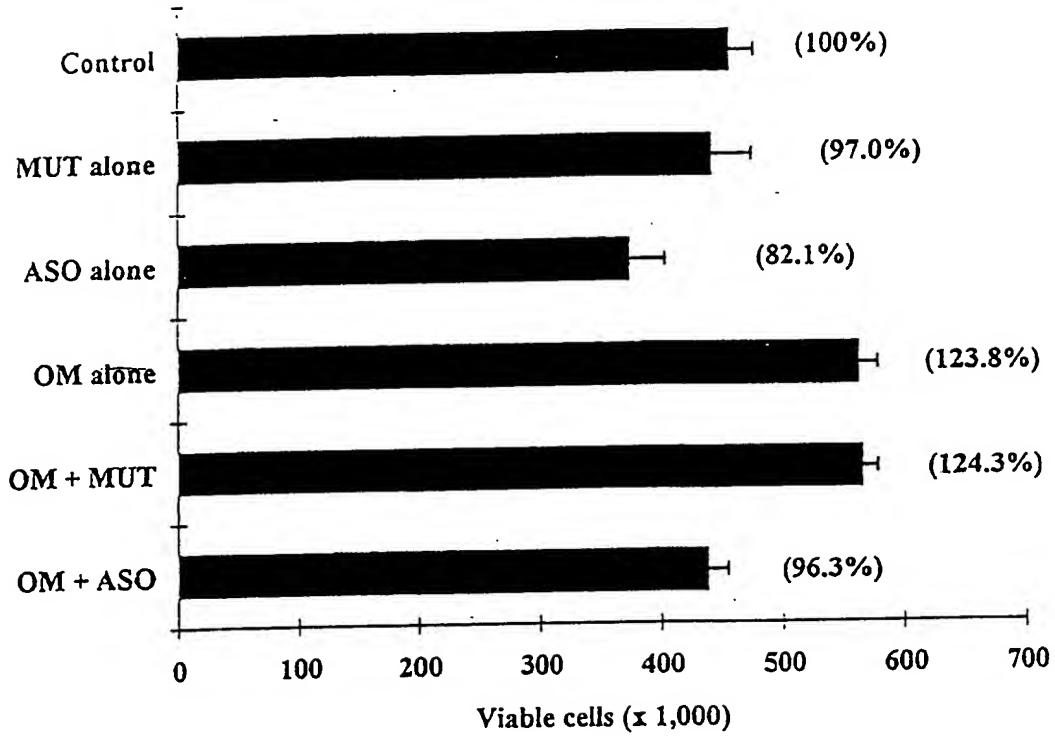
Control
MUT alone
ASO alone
OM alone
OM + MUT
OM + ASO
(100%)
(97.0%)
(82.1%)
(123.8%)
(124.3%)
(96.3%)
0
100
200
300
400
500
600
700
Viable cells (x 1,000)

FIG. 4

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/IT 98/00169

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 45/00, A61K 38/20 // C12N 15/11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, PAJ, CA, REGISTRY, EMBL/PIR/SWISSPROT/GENESEQ, MEDLINE, BIOSIS, EMBASE, DBA, DERWENT DRUG FILE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	EP 0800829 A1 (CHUGAI SEIYAKU KABUSHIKI KAISHA), 15 October 1997 (15.10.97), page 2, line 54 - line 59; page 3, line 9 - line 11, page 3, line 18 - line 22; page 3, line 34 - line 38; page 6, line 54 - line 56; page 9, line 2 - line 23	1-7
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X	WO 9620728 A1 (CHUGAI SEIYAKU KABUSHIKI KAISHA), 11 July 1996 (11.07.96), see the references in the corresponding EP 0 800 829 A1	1-7
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 Sept 1998	14 10. 1998
Name and mailing address of the ISA: European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+ 31-70) 340-3016	Authorized officer CAROLINA PALMCRANTZ

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IT 98/00169

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, File 377, Derwent Drug File, Dialog accession no. 00699459, Derwent accession no. 96-29791, Borsellino N. et al: "Therapeutic agents II (genes, gene components, antireceptors). Effects of an IL-6 receptor super-antagonist (IL-6R SAnt 7) on the growth and sensitivity to etoposide (VP-16) of the hormone resistant and IL-6-secreting prostate tumor PC-3 cell line", Proc.Am.Assoc.Cancer Res.37,87 Meet.,410-411	1-7
	--	
A	WO 9634104 A1 (ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P. ANGELETTI S.P.A.), 31 October 1996 (31.10.96)	1-7
	--	
A	EP 0747480 A1 (GEN-PROBE INCORPORATED), 11 December 1996 (11.12.96)	4
	--	

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.
PCT/IT 98/00169

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0800829 A1	15/10/97	AU 4356896 A	24/07/96
		CH 549013 A	15/05/74
		CH 561171 A	30/04/75
		DE 2058532 A,B,C	03/02/72
		DE 2065365 A,B,C	17/05/73
		FR 2073434 A,B	01/10/71
		FR 2215419 A,B	23/08/74
		GB 1349357 A	03/04/74
		GB 1349358 A	03/04/74
		NL 145842 B	15/05/75
		NL 160244 B,C	15/05/79
		NL 160551 B,C	15/06/79
		NL 7017367 A	02/06/71
		NL 7500209 A	29/04/75
		NL 7500210 A	29/04/75
		SE 378102 B,C	18/08/75
		SE 411900 B,C	11/02/80
		US 3872087 A	18/03/75
		US 3954227 A	04/05/76
		US 4018824 A	19/04/77
		US 4360464 A	23/11/82
		CA 2209124 A	11/07/96
		CN 1174507 A	25/02/98
		JP 8231433 A	10/09/96
		WO 9620728 A	11/07/96
WO 9620728 A1	11/07/96	AU 4356896 A	24/07/96
		CA 2209124 A	11/07/96
		CN 1174507 A	25/02/98
		EP 0800829 A	15/10/97
		JP 8231433 A	10/09/96
WO 9634104 A1	31/10/96	EP 0822986 A	11/02/98
		IT 1276555 B	03/11/97
		IT RM950273 A,U,V	28/10/96
		JP 10506538 T	30/06/98
EP 0747480 A1	11/12/96	AU 5753196 A	30/12/96
		US 5674995 A	07/10/97
		US 5780612 A	14/07/98
		WO 9640908 A	19/12/96
		US 5747470 A	05/05/98